REMARKS

Claim 19 has been canceled without prejudice or disclaimer. Claims 1, 31, and 32 have been amended, and are now reciting polynucleotide sequences of interest "having or encoding a desired characteristic". Support for this amendment is found in the original specification, e.g., on page 10, lines 21-24. Claim 3 was amended solely to correct a grammatically incorrect use of plural.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Rejection of Claims 1-9, 11, 13-18, 20-21, 30-32 under 35 U.S.C. 103

Claims 1-9, 11, 13-18, 20-21, 30-32 are rejected under 35 U.S.C. 103 as being unpatentable over Christensen (WO 98/01470) in view of Aleksenko et al (Mol. Microbiol. (1996) 19(3), 565-574) and Dalboge et al (Mol. Gen. Genet (1994) 243-260). This rejection is respectfully traversed.

The present invention relates to "*a method for screening a library of polynucleotide sequences of interest having or encoding a desired characteristic in filamentous fungal cells*". Further, the invention specifies, that the library of the invention comprises "*vectors in the population that vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest*".

In summary, the invention relates to the screening of a polynucleotide variant-library, wherein all the nucleotides have or encode a variant of the desired characteristic; the purpose of the invention is to screen or select for one or more superior transformant(s), wherein these are identified by the use of appropriate screening or selecting assay(s).

Screening a variant library in a filamentous fungus using the vector-based expression system recited in the claims had not been disclosed or even suggested before the present invention. Indeed, a uniform plasmid copy number throughout a population of filamentous fungal cells could not be achieved at the time of the invention, and uniform gene-expression was not possible in filamentous fungal cells without chromosomal integration. Consequently, it was not a straightforward task in the art to do comparative studies on very similar variants from a library of variants in filamentous fungi.

Cloning in yeast and filamentous fungi was well-known in the art, as were plasmid-borne expression systems, and plasmid/chromosome based gene-expression studies. That much is clear from the disclosure of Christensen et al.

Plasmids are often referred to as extrachromosomal elements, as independently replicating, or as autonomously maintained. However, not many truly autonomously maintained (i.e. independently replicating) plasmid systems had been described for filamentous fungi at the time of filing this application, and none had been put to use in the method of this invention. The Examiner cites a publication by Aleksenko et al. (1996) wherein autonomously replicating AMA1-based plasmids are shown.

The inventors cited (in the background section of this application) a more recent publication on this topic, which was co-authored by Aleksenko (Fungal Genetics and Biology (1997) 21:373-387). In the cited Aleksenko (1997) reference, it is described how recombination had been utilized in order to facilitate gene cloning by co-transforming an AMA1-based vector with a prepared gene library on an integrating vector which normally transforms at a lower frequency. The vectors were presumably converted *in vivo* by recombination to an AMA1-based autonomous replicating vector. This was demonstrated by cloning *adC* and *adD* from an *A. nidulans* gene (genomic) library by a classical mutation-complemention screen.

A person skilled in the art would recognize the use of the term "gene library" or "gene bank" in either Aleksenko reference as meaning a library of sequences that in their totality represent the genome of the organism, from which it was derived, in this case, from *A. nidulans*. Screening a genomic library for a gene of interest, such as, is done for *adC* by Aleksenko et al., and screening a "library of variants of a polynucleotide" as defined in the present invention, are two completely different tasks that require very different methodological approaches. The first is akin to finding the proverbial needle in the haystack by using a magnet, whereas the present invention is akin to looking for a number of particularly sharp needles in a stack of needles.

To the point, Aleksenko et al. certainly do not in either reference disclose a variant gene library or "a library of variants of a polynucleotide" as defined in the present invention (p.5, l.8-28). Nowhere is it disclosed or suggested in the Aleksenko et al. references of how to establish a gene library, be it a genome or a variant library, directly in a vector comprising a fungal replication initiating sequence such as AMA1, before transforming the fungal host cell. Nor is a method for screening a variant library of the present invention for a sequence of interest mentioned anywhere therein. Despite the fact that entire sections of the Aleksenko (1997) publication are dedicated to listing various potential applications of AMA1-like sequences, including applications in gene

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cloning and in gene expression studies, it does not even touch upon variant libraries at all or the screening of such, according to the present invention.

It is therefore clear that there would have been no motivation for one skilled in the art to begin constructing a completely new type of vectors based on the Aleksenko et al. publications on AMA1. Indeed, there was no way to predict that prepared AMA1-based vectors, not meant for co-transformation (as Aleksenko et al. promoted in all their publications), would have such ideally suited properties for the method of this invention: uniform copy number within the cell population combined with long-term stability.

Therefore, Christensen (WO 98/01470) in view of Aleksenko et al (Mol. Microbiol. (1996) 19(3), 565-574) and Dalboge et al (Mol. Gen. Genet (1994) 243-260) do not suggest the present invention.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 1-9, 11, 13-18, 20-21, 30-32 under 35 U.S.C. 112

Claims 1-9, 11, 13-18, 20-21, 30-32 are rejected under 35 U.S.C. 112 as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant's regards as the invention. The Examiner has maintained this objection for reason of record, that is, because "the claims do not recite how the transformants are selected". This rejection is respectfully traversed.

As is clear from the above discussion, the instant invention relates to "a method for screening a library of polynucleotide sequences of interest having or encoding a desired characteristic in filamentous fungal cells".

How the transformants are selected depends on what the desired characteristic is in each embodiment of the invention. Any appropriate selection procedure is clearly applicable and is covered by the claims. For example, if the desired characteristic is improved thermostability of an enzyme, then, e.g., an activity-based assay at high temperature can be employed.

Plainly, it is improper and an undue restriction to limit the claims to a single or preferred selection method when the invention contemplates and is broadly applicable to the use of any appropriate selection method, and when such selection methods are clearly well within the skill of an artisan practicing the claimed invention.

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The claims are not indefinite for lacking an essential step as the phrase "selecting or screening for one or more transformants expressing the desired characteristic" is not so narrow as to encompass only a particular assay, and an artisan would clearly understand that this phrase encompasses the many procedures well-known in the art for identifying one or more transformants expressing a desired characteristic.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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Attorney Docket No.: 5579.210-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jesper Vind Confirmation No: 1334

Serial No.: 09/426,038 Group Art Unit: 1627

Filed: October 25, 1999 Examiner: P. Ponnaluri

For: Methods Of Constructing And Screening A DNA Library Of Interest In Filamentous Fungal Cells

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE CLAIMS:

The claims have been amended as follows:

1. (Amended.) A method of screening a library of polynucleotide sequences of interest having or encoding a desired characteristic in filamentous fungal cells, wherein the method comprises:

(a) transforming the fungal cells with a population of DNA vectors, wherein each vector comprises:

(iii) a fungal selection marker polynucleotide sequence and a fungal replication initiating polynucleotide sequence, wherein the marker and the replication initiating sequence do not vary within the population; and wherein the replication initiating sequence is a nucleic acid sequence selected from the group consisting of: (1) a replication initiating sequence having at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3, and is capable of initiating replication; and (2) replication initiating sequence which hybridises under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or (ii) the respective complementary strands, wherein the low stringency conditions are defined by prehybridization and hybridization at 42°C in 5x SSPE, 0.3% SDS, 200 mg/ml sheared and denatured salmon sperm DNA, and 25% formamide, and wash conditions are defined at 50°C for 30 minutes in 2x

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SSC, 0.2% SDS; and

(iv) a polynucleotide sequence of interest, wherein there are vectors in the population that vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest;

(b) cultivating the cells in the presence of an effective amount of a selective agent or the absence of an appropriate selective agent;

(c) selecting or screening for one or more transformants expressing [a] the desired characteristic; and

(d) isolating the transformant(s) of interest.

2. (Unchanged.) The method according to claim 1, wherein the library of polynucleotide sequences of interest is prepared by random mutagenesis or naturally occurring allelic variations of at least one parent polynucleotide sequence having or encoding a biological activity or function of interest.

3. (Amended.) The method of claim 1, wherein the polynucleotide sequence further comprises a control sequence[s].

4. (Unchanged.) The method according to claim 1, wherein the polynucleotide sequence of interest encodes a hormone, an enzyme, a receptor or a portion thereof, an antibody or a portion thereof, or a reporter, or a regulatory protein.

5. (Unchanged.) The method of claim 4, wherein the enzyme is an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, or a ligase.

6. (Unchanged.) The method according to claim 4, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, a proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

7. (Unchanged.) The method according to claim 3, wherein the control sequence is an

enhancer sequence, a leader sequence, a polyadenylation sequence, a propeptide sequence, a promoter, a replication initiation sequence, a signal sequence, a transcriptional terminator or a translational terminator.

8. (Unchanged.) The method of claim 7, wherein the promoter is derived from the gene encoding *Aspergillus oryzae* TAKA amylase, NA2-tpi and *Aspergillus niger* or *Aspergillus awamori* glucoamylase.

9. (Unchanged.) The method according to claim 1, wherein the selection marker polynucleotide sequence is selected from the group of genes which encode a product which is responsible for one of the following: resistance to biocide or viral toxicity, resistance to heavy metal toxicity, prototrophy to auxotrophs.

11. (Unchanged.) The method of claim 9, wherein the selection marker polynucleotide sequence is a gene selected from the group consisting of *argB* (ornithine carbamoyltransferase), *amdS* (acetamidase), *bar* (phos-hinothricin acetyltransferase), *hemA* (5-aminolevulinate synthase), *hemB* (porphobilinogen synthase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *prn* (proline permease), *pyrG* (orotidine-5'-phosphate decarboxylase), *pyroA*, *riboB*, *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase).

13. (Unchanged.) The method of claim 1, wherein the replication initiating polynucleotide sequence has at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3.

14. (Unchanged.) The method of claim 1, wherein the replication initiating polynucleotide sequence is obtained from a filamentous fungal cell.

15. (Unchanged.) The method of claim 14, wherein the filamentous fungal cell is a strain of *Aspergillus*.

16. (Unchanged.) The method of claim 15, wherein the strain of *Aspergillus* is obtained from a

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strain of *A. nidulans*.

17. (Unchanged.) The method of claim 1, wherein the replication initiating polynucleotide sequence has the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

18. (Unchanged.) The method of claim 2, wherein the polynucleotide sequence of interest was created by mutagenesis, by random mutagenesis, by use of a physical or chemical mutagenizing agent, by use of a doped oligonucleotide, by DNA shuffling, by subjecting the nucleic acid sequence to PCR generated mutagenesis, or by use of any combination thereof.

20. (Unchanged.) The method according to claim 1, wherein the filamentous fungal cell transformed with the population of DNA vectors is a cell of a strain of *Acremonium, Aspergillus, Coprinus, Fusarium, Humicola, Mucor, Myceliopthora, Neurospora, Penicillium, Thielavia, Tolypocladium* or *Trichoderma*.

21. (Unchanged.) The method according to claim 20, wherein the cell is an *Aspergillus oryzae, Aspergillus niger, Aspergillus nidulans, Coprinus cinereus, Fusarium oxysporum*, or *Trichoderma reesei* cell.

30. (Unchanged.) The method of claim 1, wherein the polynucleotide sequence of interest is a control sequence.

31. (Amended.) A method of constructing a library of polynucleotide sequences of interest <u>having or encoding a desired characteristic</u> in filamentous fungal cells, wherein the method comprises:

(a) transforming the fungal cells with a population of DNA vectors, wherein each vector comprises:

(i) a fungal selection marker polynucleotide sequence and a fungal replication initiating polynucleotide sequence, wherein the marker and the replication initiating sequence do not vary within the population; and wherein the replication initiating sequence is a nucleic acid sequence selected from the group consisting of: (1) a replication initiating sequence having at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP

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computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3, and is capable of initiating replication; and (2) replication initiating sequence which hybridises under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or (ii) the respective complementary strands, wherein the low stringency conditions are defined by prehybridization and hybridization at 42°C in 5x SSPE, 0.3% SDS, 200 mg/ml sheared and denatured salmon sperm DNA, and 25% formamide, and wash conditions are defined at 50°C for 30 minutes in 2x SSC, 0.2% SDS; and

(ii) a polynucleotide sequence of interest <u>having or encoding a desired characteristic</u>, wherein there are vectors in the population that vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest;

(b) cultivating the cells in the presence of an effective amount of a selective agent or the absence of an appropriate selective agent.

32. (Amended.) The method of claim 31, further comprising the steps of:

(c) selecting or screening for one or more transformants expressing [a] <u>the</u> desired characteristic; and

(d) isolating the transformant(s) of interest.

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